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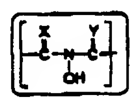
(54) Title: MULTICOMPONENT SYSTEM FOR CHANGING, REDUCING OR BLEACHING LIGNIN, LIGNIN-CONTAINING MATERIALS OR SIMILAR SUBSTANCES AS WELL AS PROCESSES FOR ITS APPLICATION

(54) Bezeichnung: MEHRKOMPONENTENSYSTEM ZUM VERÄNDERN, ABBAU ODER BLEICHEN VON LIGNIN, LIGNINHALTIGEN MATERIALIEN ODER ÄHNLICHEN STOFFEN SOWIE VERFAHREN ZU SEINER ANWENDUNG

(57) Abstract



(A)

This invention concerns a multicomponent system for changing, reducing or bleaching lignin, lignin-containing materials or similar substances containing a) optionally, at least one oxidation catalyst and b) at least one suitable oxidant and c) at least one mediator characterized by the fact that the mediator is selected from the cyclical N-hydroxy compounds with at least one optionally substituted five- or six-member ring containing the structure named in formula (A), as well as its salts, ethers or esters, where X and Y are the same or different and mean O, S, or NR^1, where R^1 means a hydrogen, hydroxy, formyl, carbamoyl, sulfono radical, an ester or salt of the sulfono radical, a sulfamoyl, nitro, amino, phenyl, aryl C_1-C_5-alkyl, C_1-C_{12}-alkyl, C_1-C_5-alkoxy, C_1-C_{10}-carbonyl, carbonyl-C_1-C_6-alkyl, phospho, phosphono, phosphonooxy radical, ester or salt of the phosphonooxy radical, where the carbamoyl, sulfamoyl, amino and phenyl radicals can be unsubstituted or singly or multiply substituted with radical R^2, and the aryl-C_1-C_5-alkyl, C_1-C_{12}-alkyl, C_1-C_5-alkoxy, C_1-C_{10}-carbonyl, carbonyl-C_1-C_6-alkyl radicals can be saturated or unsaturated, branched or unbranched, where R^2 is the same or different and represents a hydroxy, formyl, carboxy radical, an ester or salt of the carboxy radical, a carbamoyl, sulfono ester or salt of the sulfono radical, a sulfamoyl, nitro, amino-, phenyl, C_1-C_5-alkyl, C_1-C_5-alkoxy radical.

(57) Zusammenfassung

Mehrkomponentensystem zum Verändern, Abbau oder Bleichen von Lignin, ligninhaltigen Materialien oder ähnlichen Stoffen enthaltend a. ggf. mindestens einen Oxidationskatalysator und b. mindestens ein geeignetes Oxidationsmittel und c. mindestens einen Mediator, dadurch gekennzeichnet, daß der Mediator ausgewählt ist aus der Gruppe cyclischer N-Hydroxyverbindungen mit mindestens einem ggf. substituierten fünf- oder sechsgliedrigen Ring enthaltend die in Formel (A) genannte Struktur, sowie deren Salze, Ether oder Ester, wobei X und Y gleich oder verschieden sind, und O, S, oder NR^1 bedeuten, wobei R^1 Wasserstoff-, Hydroxy-, Formyl-, Carbamoyl-, Sulfonorest, Ester oder Salz des Sulfonorests, Sulfamoyl-, Nitro-, Amino-, Phenyl-, Aryl-C_1-C_5-alkyl-, C_1-C_{12}-Alkyl-, C_1-C_5-Alkoxy-, C_1-C_{10}-Carbonyl-, Carbonyl-C_1-C_6-alkyl-, Phospho-, Phosphono-, Phosphonooxyrest, Ester oder Salz des Phosphonooxyrests bedeutet, wobei Carbamoyl-, Sulfamoyl-, Amino- und Phenylreste unsubstituiert oder ein- oder mehrfach mit einem Rest R^2 substituiert sein können und die Aryl-C_1-C_5-alkyl-, C_1-C_{12}-Alkyl-, C_1-C_5-Alkoxy-, C_1-C_{10}-Carbonyl-, Carbonyl-C_1-C_6-alkyl-Reste gesättigt oder ungesättigt, verzweigt oder unverzweigt sein können und mit einem Rest R^2 ein- oder mehrfach substituiert sein können, wobei R^2 gleich oder verschieden ist und Hydroxy-, Formyl-, Carboxyrest, Ester oder Salz des Carboxyrests, Carbamoyl-, Sulfono-Ester oder Salz des Sulfonorests, Sulfamoyl-, Nitro-, Amino-, Phenyl-, C_1-C_5-Alkyl-, C_1-C_5-Alkoxyrest bedeutet.

A multicomponent system for changing, reducing or bleaching of lignin, lignin-containing materials or similar substances as well as processes for its application

The present invention concerns a multicomponent system for changing, reducing or bleaching of lignin, lignin-containing materials or similar substances as well as processes for its application.

At present the principal processes used for cellulose manufacturing are the sulfate and the sulfite processes. With both processes, cellulose is produced under boiling conditions and at high pressures. The sulfate process works by adding NaOH and Na_2S, whereas in the sulfite process $Ca(HSO_3)_2 + SO_2$ is used.

All processes have as their principal purpose, the removal of the lignin from plant materials (wood or annual plants).

The lignin which, with cellulose and hemicellulose, forms the main component of the plant material (stalk or trunk) must be removed, because otherwise it is not possible to manufacture paper that does not age and is highly resistant to stress.

The processes for manufacturing of wood pulp employ stone-grinders (groundwood) or refiners (TMP) defiberize the wood by grinding after appropriate preliminary treatment (chemical, thermal or chemical-thermal).

This wood pulp still contain a significant fraction of lignin. It is used principally for the manufacture of newspapers, magazines, etc..

For some years, ways of using enzymes for the reducing of lignin have been explored. The mode of action of such lignolytic systems has only just been understood following the success in gaining sufficient amount of enzymes derived from

the white rot fungus *phanerochaete chrysoporium*. This was done in optimal growth conditions with appropriate additives. By doing this, the lignin peroxidases and the manganese peroxidases were discovered which were previously unknown. Since *phanerochaete chrysoporium* is a very effective reducting agent of lignin, attempts to isolate its enzymes and to use them in a purified form for the lignin reduction had been tried. This had, however, not been successful, because it turned out that the enzymes lead to a repolymerisation of the lignin and not to its reduction.

Something similar applies to other species of lignolytical enzymes like laccases which reduce the lignin with the help of oxygen instead of hydrogen peroxide. It has been established that similar processes happen in any event. Actually there are radicals formed that react again with each other and consequently lead to polymerization.

Therefore the only processes that work today involve in-vivo systems (fungus based). The main foci of efforts to optimize the processes are the so called biopulping and the biobleaching techniques.

Biopulping is the treatment of wood chips by living systems based on fungus.

Two methods of application exist:

1. A preliminary treatment of wood chips before refining or grinding in order to save energy while manufacturing the wood pulp (i.e. TMP or groundwood).

A second advantage is that often there is an improvement in the mechanical properties of the pulp. A disadvantage is the loss in quality of the final white color.

2. A preliminary treatment of the wood chips (softwood/hardwood) before cooking the cellulose (kraft process and sulfite process).

Here a reduction of cooking chemicals together with the improvement of cooking capacity and "extended cooking" is the aim.

The achievement of an improved kappa reduction after the cooking (compared with cooking without a preliminary treatment) is an advantage, also.

The clear disadvantages of the processes are the long times of treatment (several weeks) and in particular the unsolved danger of contamination during the treatment if it is carried out without sterilization of the wood chips, a process which is uneconomic.

The biobleaching process likewise works by employing in-vivo systems. The cooked cellulose (derived from softwood/hardwood) is inoculated with fungus and treated for days or even weeks. Only after this long time of treatment is a significant lowering of the kappa number and an improvement in whiteness seen. This makes the process uneconomic for implementation within common bleaching sequences.

Another application often carried out using immobilized fungus based systems, is the treatment of waste caused by cellulose production, especially removing the color from the effluent of bleaching factories and reducing the AOX (reducing of chlorine-compounds in the waste caused by chlorine stages and chlorine dioxide stages in the bleaching process).

In addition it is common to use hemicellulases such as xylanases and mannanases as "bleach boosters".

These enzymes are used principally to reduce the amount of precipiated xylan that partly covers the remaining lignin after the cooking process. The reactivity of lignin to the bleaching chemicals (especially the chlorine dioxide) used in the

following sequences should be enhanced by reducing the xylan. The savings of bleaching chemicals that have been demonstrated in the laboratory are only partially confirmed in larger scale experiments. Therefore the type of enzyme can be classified only as an additive to the bleach.

The cofactors beside the lignolytic enzymes are assumed to be chelating substances (siderophores such as ammonia oxalate) and bio-detergents.

In application PCT/EP87/00635 a system is described which removes lignin from materials that contain lignin cellulose and simultaneously produces bleaching. It works by lignolytical enzymes from fungi with addition of reducing agents, oxidizing agents and, as mediators, phenol based compounds.

In DE 4008893C2 in addition to the red/ox-systems there were added "mimic-substances" which simulate the active site (prosthetic group) of lignolytic enzymes. In so there could be achieved a considerable improvement in performance.

In application PCT/EP92/01086 a redox cascade is used as an additional improvement. It works by phenolic and non phenolic aromatics having an "suitable" oxidizing potential.

In all three processes the limitation for industrial use is the usability at low densities of pulp (up to maximum of 4%) and in the last applications the danger of "outleaching" of metal when chelating systems are used. These systems may lead to destruction of peroxide, especially in the final stages of bleaching which use peroxids.

The processes denoted in WO/12619, WO 94/12620 and WO 94/12621 are those in which the activity of the peroxidase is enhanced by so called enhancer-substances.

The enhancer-substances are characterized in WO 94/12619 on the basis of their active half-life.

According to WO 94/12620 enhancer-substances are characterized by the formula A=N-N=B, where A and B are particularly defined cyclic residues.

According to WO 94/12620 enhancer-substances are organic chemicals that minimally contain two aromatic rings. At least one of these rings is substituted by particularly defined residues.

All three patent applications concern the "dye transfer inhibition" and the use of particular enhancer-substances together with peroxidases as a detergent-additive or as a detergent-composition in the detergent sector. In their application's description reference is made to the treatment of lignin, but experiments performed by the present applicant with substances which are explicitly revealed by the application have shown that they produce no effect on the enhancing of the peroxidases' bleaching-effects when using them for mediators.

WO 94/29510 describes a process for enzymatic delignification in which enzymes are used together with mediators. In general compounds of structure NO-, NOH- or HRNOH are demonstrated as mediators.

From the mediators demonstrated in WO 94/29510, 1-hydroxy-1H-benzotriazole (HBT) produce the best results for the removal of lignin. HBT has, however, various disadvantages:

It is only available at high prices and not in sufficient quantities.

Under conditions for delignification HTB reacts to form 1H-benzotriazole. In large amounts this compound produce

considerable pollution of the environment, because it is difficult to break down.

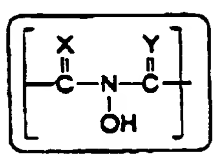
In addition to that 1-hydroxy-1H-benzotriazole under the influence of oxidizing agents (such as those used for the delignification process) reacts to produce further uncharacterized decomposition products which show undesirable strong color.

The present invention concerns a multicomponent system for changing, reducing or bleaching of lignin, lignin-containing materials or similar substances containing

a. (if applicable) at least one oxidatizing catalyst and

b. at least one suitible oxidazing agent and

c. at least one mediator characterized in so that the mediator is choosen from the group of cyclic N-hydroxy compounds with at least one (if applicable) substituted five- or sevenmembered ring containing the structure called formula A



formula A

as well as their salts, ethers or esters, where

X and Y are the same or different and represent O, S or NR^1, where

R^1 represents hydrogen-, hydroxy-, formyl-,carbamoyl-, sulfo-residue, ester or salt of the sulfo residue, sulfamoyl-, nitro-, amino-, phenyl-, aryl-C_1-C_5-alkyl-, C_1-C_{12}-alkyl-, C_1-C_5-alkoxy-, C_1-C_{10}-carbonyl-, carbonyl-C_1-C_6-alkyl, phospho-,

phosphono-, phosphonooxy-residue, ester or salt of the phosphonooxy residue,

where carbamoyl-, sulfamoyl-, amino- and phenyl-residues can be unsubstituted or substituted once or multiple with a residue R^2 and the aryl-C_1-C_5-alkyl-, C_1-C_{12}-alkyl-, C_1-C_5-alkoxy-, C_1-C_{10}-carbonyl-, carbonyl-C_1-C_6-alkyl-residues can be saturated or unsaturated, branched or unbranched and can be substituted once or multiple by a residue R^2 where

R^2 is the same or different and represents hydroxy-, formyl-, carboxy residue, ester or salt of the carboxy residue, carbamoyl-, sulfo-ester or salt of the sulfo residue, sulfamoyl-, nitro-, amino-, phenyl-, C_1-C_5-alkyl-, C_1-C_5-alkoxy-residue.

The multicomponent system in accordance with this invention contains mediators that are industrially available at more reasonable prices compared with HTB. These mediators react under the influence of oxidizing agents to compounds without a change of color spoiling the final effect. These products are completely reducible.

Preferably, the multicomponent system in accordance with this invention contains one oxidizing catalyst.

In the multicomponent system in accordance with this invention enzymes are preferably used for oxidizing catalysts. In accordance with this invention the term "enzyme" includes enzymatically active proteins, peptides or prosthetic groups of enzymes.

In the multicomponent system according to the invention oxireductases of classes 1.1.1 to 1.97 according to the International Enzyme Nomenclature, Committee of the

International Union of Biochemistry and Molecular Biology (Enzyme Nomenclature, Academic Press, Inc., 1992, pp. 24-154) may be used as an enzyme.

Preferably, enzymes of the following named classes are used:

Enzymes of class 1.1 that include all dehydrogenases having an effect on primary, secondary alcohols and semiacetals and having NAD^+ or $NADP^+$ (subclass 1.1.1), cytochromes (1.1.2), oxygen (O_2) (1.1.3), disulfide (1.1.4), quinone (1.1.5) as acceptors or that have other acceptors (1.1.99).

From this class particularly preferred enzymes are these from class 1.1.5 together with quinones to be the acceptors and these enzymes from class 1.1.3 together with oxygen to be the acceptor.

Especially preferred in this class is cellobiose: quinone-1-oxireductase (1.1.5.1).

In addition the enzymes from class 1.2 are preferred. This enzyme class (1.1.5.1) includes such enzymes that oxidize aldehydes to the corresponding acids or oxo-groups. The acceptors may be NAD^+, $NADP^+$ (1.2.1), cytochrome (1.2.2), oxygen (1.2.3), sulfide (1.2.4), iron-sulfur-proteins (1.2.5) or other acceptors (1.2.99).

The enzymes from group (1.2.3) with oxygen as acceptor are preferred in particular.

In addition the enzymes from class 1.3 are preferred. In this class enzymes are brought together that have an effect on the CH-CH-groups of the donator.

The corresponding acceptors are NAD^+, $NADP^+$ (1.3.1), cytochrome (1.3.2), oxygen (1.3.3), quinone or related compounds (1.3.5), iron-sulfur-proteins (1.3.7) or other acceptors (1.3.99).

Bilirubinoxidase (1.3.3.5) is preferred in particular.

Here the enzymes from class (1.3.3) together with oxygen as acceptor and (1.3.5) together with quinone etc. as acceptor are preferred in particular.

In addition the enzymes from class 1.4 which have an effect on $CH\text{-}NH_2$-groups of the donor are preferred.

The corresponding acceptors are NAD^+, $NADP^+$ (1.4.1), cytochrome (1.4.2), oxygen (1.4.3), disulfides (1.4.4), iron-sulfur-proteins (1.4.7) or other acceptors (1.4.99).

In particular the enzymes from class 1.4.3 with oxygen as acceptor are preferred.

In addition the enzymes from class 1.5 are preferred which have an effect on the CH-NH-groups of the donor. The corresponding acceptors are NAD^+, $NADP^+$ (1.5.1), oxygen (1.5.3), disulfides (1.5.4), quinones (1.5.5) or other acceptors (1.5.99).

Here enzymes with oxygen (O_2) (1.5.3) and with quinones (1.5.5) as acceptors are preferred as well.

In addition the enzymes from class 1.6 which have an effect on NADH or NADPH are preferred.

Here the acceptors are $NADP^+$ (1.6.1), haem proteins (1.6.2), disulfide (1.6.4), quinone (1.6.5), NO_2-groups (1.6.6), and a flavine (1.6.8) or some other acceptors (1.6.99).

Here the enzymes from class 1.6.5 with quinone as acceptor are preferred in particular.

In addition the enzymes from class 1.7 are preferred which act as a donator on other NO_2-compounds and which have cytochrome (1.7.2), oxygen (O_2) (1.7.3), iron-sulfur-proteins (1.7.7) or others (1.7.99) as acceptors.

Here the enzymes from class 1.7.3 with oxygen as acceptor are preferred in particular.

In addition the enzymes from class 1.8 are preferred which act as a donator on sulfur-groups and which have NAD^+, $NADP^+$ (1.8.1), cytochrome (1.8.2), oxygen (O_2) (1.8.3), disulfides (1.8.4), quinones (1.8.5), iron-sulfur-proteins (1.8.7) or others (1.8.99) as acceptors.

The class 1.8.3 with oxygen (O_2) and (1.8.5) with quinones as acceptors is preferred in paritcular.

In addition the enzymes from class 1.9 are preferred which act as a donator on haem groups and which have oxygen (O_2) (1.9.3), NO_2-compounds (1.9.6) and others (1.9.99) as acceptors.

Here the group 1.9.3 with oxygen (O_2) as acceptor is preferred in particular (cytochromeoxidases).

In addition the enzymes from class 1.12 are preferred which act as a donator on hydrogen.

The acceptors are NAD^+, $NADP^+$ (1.12.1) or others (1.12.99).

In addition to that the enzymes from class 1.13 and 1.14 are preferred (oxigenases).

In addition preferred enzymes are that from class 1.15 which act as a acceptor on superoxide-radicals.

Here the superoxide-dismutase is preferred in particular.(1.15.1.1).

In addition the enzymes of class 1.16 are preferred.

NAD^+ or $NADP^+$ (1.16.1) or oxygen (O_2) (1.16.3) act as acceptors.

Here the enzymes from class 1.16.3.1 are preferred in particular (peroxidase, i.e. ceruloplasmine).

Enzymes which are additionally preferred are these which belong to group 1.17 (effect on CH_2-groups which are oxidized to -CHOH-), 1.18 (effect on reduced ferredoxin as donor), 1.19 (effect on reduced flavodoxine as donor) and 1.97 (other oxireductases).

In addition the enzymes from group 1.11 are especially preferred which act on a peroxide as acceptor. This only subclass (1.11.1) contains the peroxidases.

Here the cytochrome-C-peroxidases (1.11.1.5), catalases (1.11.1.6), the peroxidases (1.11.1.6), the iodide-peroxidases (1.11.1.8), the glutathione-peroxidases (1.11.1.9), the chloride-peroxidases (1.11.1.10), the L-ascorbate-peroxidase (1.11.1.9), the phospholipid-hydroperoxide-glutathione-peroxidase (1.11.1.12), the manganese-peroxidase (1.12.1.13), the diarylpropane-peroxidase (ligninase, lignin-peroxidase) (1.11.1.14) are especially preferred.

The enzymes from class 1.10 which act on biophenols and related compounds are very particularly preferred. They catalyze oxidization of biophenols and ascarbates. NAD^+, $NADP^+$ (1.10.1),

cytochrome (1.10.2), oxygen (1.10.3) or others (1.10.99) act as acceptors.

Of these, however, the enzymes from class 1.10.3 with oxygen (O_2) as acceptor are preferred.

Of these classes' enzymes the enzymes catechol oxidase (tyrosinase) (1.10.3.1), L-ascorbate oxidase (1.10.3.3), o-aminophenol oxidase (1.10.3.4) and laccase (benzenediole: oxigene oxireductase) (1.10.3.2) are preferred, where laccases (benzenediole: oxigene oxireductases) (1.10.3.2) are preferred in particular.

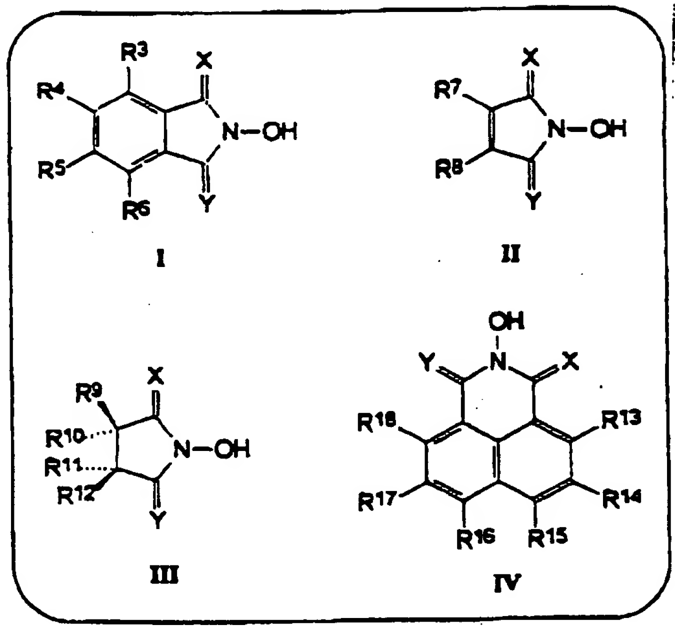
The enzymes mentioned are for sale or produced by standard methods. Plants, animal cells, bacteria and fungi (for example) can be considered as organisms producing the enzyme. In principle, both naturally existing organisms and organisms changed by genetic engineering can produce of enzymes. Parts of unicellular or multicellular organisms are conceivable as producers of enzyme just as well, especially cell cultures, also.

White rot fungi such as *pleurotus, phlebia* and *trametes* are for example used for the especially preferred enzymes such as those from groups 1.11.1 and epsecially 1.10.3, in particular for the production of laccanases.

The multicomponent system in accordance with this invention contains at least one oxidizing agent. For example air, oxygen, ozone, H_2O_2, organic peroxides, peracids such as peracetic acid, performic acid, persulfuric acid, pernitric acid, metachlorineperoxibenzoic acid, perchloride acid, perborates, peracetates, persulfates, peroxides or oxygen species and their radicals such as $OH^\bullet$, $OOH^\bullet$, singlet oxygen, superoxide ($O_2^{\bullet-}$), ozonide, dioxygenyl-cation (O_2^+), dioxiranes, dioxetanes or Fremy radicals can be used as oxidizing agent.

Such oxidizing agents are preferably used which can be either generated by corresponding oxidoreductases i.e. dioxiranes from laccases plus carbonyls or which can chemically regenerate or react directly with the mediator.

The multicomponent system in accordance with this invention includes at least one compound of the general formula I, II, III or IV as mediator (component c),

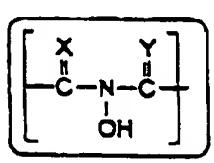


where X, Y, have the meanings previously mentioned and the residues R^3-R^{18} are the same or different and the halogen residue, carboxy residue, salt or ester of a carboxy residue or the meanings mentioned for R^1,

where R^9 and R^{10} respectively R^{11} and R^{12} cannot represent hydroxy- or amino-residue at same time and

(if applicable) each two of the substitutes R^3-R^6, R^7-R^8, R^9-R^{12}, R^{13}-R^{18} can be connected to a ring -B-, where -B- has one of the following meanings:

$(-CH=CH)-_n$ with n = 1 to 3, -CH=CH-CH=N- or



formula A

and where (if applicable) the residues R^9-R^{12} can be connected by one or two bridging elements -Q-, where -Q- is the same or different and have one of the following meanings: -O-, -S, $-CH_2-$, $-CR^{19}=CR^{20}-$;

where R^{19} and R^{20} can be the same or different and have the same meaning as R^3.

The compounds of the general formulas I, II, III or IV, where X and Y represent O or S, are preferred, as mediators, in particular.

Examples for such compounds are N-hydroxy-phthalimide and (if applicable) substituted N-hydroxy-phthalimide-derivatives, N-hydroxymaleimide and (if applicable) substituted N-hydroxymaleimide-derivatives, N-hydroxy-naphthal acid, imide-derivatives,N-hydroxylsuccinimide-derivatives, preferably such, where the residues R^9-R^{12} are polycyclically connected.

N-hydoxyphthalimide is especially preferred as mediator (component c of the multicomponent system in accordance with this invention).

Compounds of formula I which are suitable mediator are for example:

N-hydroxyphthalimide,
N-hydroxy-benzene-1,2,4-tricarboxylic acid, imide,
N-N'-dihydroxy-pyromellit acid, dimide,
N,N'-dihydroxy-benzophenone-3,3',4,4'-tetracarboxylic acid, diimide.

Compounds of formula II which are suitable mediators are for example:

N-hydroxymaleimide,
pyridin-2,3-dicarboxylic-acid-N-hydroxyimide.

Compounds of formula III which are suitable mediators are for example:

N-hydroxysuccinimide
N-hydroxytartric acid, imide,
N-hydroxy-5-norbornene-2,3-dicarboxylic acid, imide,
exo-N-hydroxy-7-oxabicyclo[2.2.1]-hept-5-en-2,3-dicarboximide,
N-hydrocy-cis-cyclohexene-1,2-dicarboximide,
N-hydroxy-cis-4-cyclohexene-1,2-dicarboxylic acid, imide.

A Compound of formula VI which is a suitable mediator is for example:

N-hydroxynaphthal-acid, imide, sodium salt.

A compound with a sixmembered ring containing the structure of formula A which is a suitable mediator is for example:

N-hydroxyglutarimide.

The above mentioned exemplary compounds are suitable, as mediators, in form of their salts or esters, also.

The invention concerns the use of substances, also, which in accordance with the invention are suitable to be mediators for changing, reducing or bleaching of lignin, lignin-containing materials or similar substances.

The effectiveness of the multicomponent system in changing, reducing or bleaching of lignin, lignin-containing materials or similar substances often is enhanced again if there are Mg^{2+} ions beside the mentioned components. The Mg^{2+} ions can, for

example, be used as a salt, such as, eg. $MgSO_4$. The concentration is in the range from 0.1 - 2 mg/g of lignin-containing material, especially in the range 0.2 - 0.6 mg/g.

In some cases an enhancement of effectiveness of the multicomponent system in accordance with this invention can be achieved in that the multicomponent system may contain complex formers such as, eg. ehtylenediaminetetraacetic acid (EDTA), diethylenetriaminpentaacetic acid (DTPA), hydroxyethylenediamintriacetic acid (HEDTA), dithylenetriaminpentamethylenephosphoric acid (DTMPA), nitrilotriacetic acid (NTA), polyphosphoric acid (PPA), etc.. The concentration is in the range from 0.2 - 5 mg/g of lignin-containing materials, at 1 - 3 mg, preferably.

The use of the multicomponent system in accordance with this invention in a process for treatment of lignin is for example done by mixing the particularly chosen components a) to c) in accordance with claim 1 simultaneously or in arbitrary order with an aqueous suspension of lignin-containing materials.

Preferably, a process using the multicomponent system in accordance with this invention is performed in the presence of oxygen or air at normal pressure up to 10 bar and in a pH-range from 2 to 11, at temperatures from 20 to 95 °C, preferably 40 - 95 °C, and a pulp density from 0.5 to 40 %.

An unusual and surprising result for the use of enzymes to bleach cellulose is that increasing the pulp density make it possible to enhance the kappa reduction while using the multicomponent system in accordance with this invention.

For economical reasons at pulp densities from 6 to 30 weight-%, especially from 9 to 15 weight-%, a process in accordance with this invention is preferred.

Surprisingly, it is further shown that for some celluloses an acidic washing (pH 2 to 6, preferably 4 to 5) or Q-stage (pH-value 2 to 6, preferably 4 to 5) used before the enzyme-mediator stage leads to a significant reduction of the kappa number compared to treatment without this special preliminary treatment. In the Q-stage substances are used as chelating agents which are common for those purposes (such as eg. EDTA, DPTA). They are preferably used in concentrations from 0.1 %/t to 1 %/t and preferred from 0.1 %/t to 0.5 %/t in particular.

In the process in accordance with the invention 0.01 to 10.000 units (U) enzyme per g of the lignin-containing material are preferably used. In particular 0.1 to 100 are preferred; especially preferred are 1 to 40 U enzyme per g lignin-containing material. (1 U corresponds to a conversion of 1 µmol 2,2'-azino-bis(3-thyl-benzothiazoline-6-sulfonic-acid-diammonia salt) (ABTS)/min/ml enzyme)

For the process in accordance with this invention 0.01 mg to 100 mg oxidizing agent per g lignin-containing materials are preferably used. 0.01 mg to 50 mg oxidizing agent per g lignin-containing materials are preferably used in particular.

For the process in accordance with this invention 0.01 mg to 80 mg mediator per g lignin-containing materials are preferably used. 0.5 mg to 40 mg mediator per g lignin-containing materials are preferably used in particular.

Simultaneously reducing agents can be added which, together with the existing oxidizing agents, serve to the setting of a particular redox potential.

Sodium-bisulfite, sodium-dithionite, ascorbic acid, thiocompounds, mercaptocompunds or glutathion etc. can be used as reducing agents.

The reaction goes off for example for laccase under air or oxygen supply or excess pressure of oxygen or air, for peroxidases (i.e. lignin peroxidases, manganese peroxidases) with hydrogen peroxide. Here for example oxygen can also be generated in-situ by hydrogen peroxide + catalase and hydrogen peroxide by glucose + glucose oxidase or other systems.

Additionally, radical formers or radical caotors (capture of $OH^{\bullet}$ or $OOH^{\bullet}$ radicals for example) can be added to the system. Those can improve interplay within re/ox and radical mediators.

Other metal salts can be added to the reaction solution, also.

These are important in the interplay with chelating agents as radical formers or red/ox centers. These salts form cations in reaction solution. Such ions are among others, Fe^{2+}, Fe^{3+}, Mn^{2+}, Mn^{3+}, Mn^{4+}, Cu^{2+}, Ca^{2+}, Ti^{3+}, Cer^{4+}, Al^{3+}.

The chelating substances that are in the solution can in addition to that serve as mimic substances to enzymes, for example to laccases (copper complexes) or to lignin oxidases or manganese oxidases (haem complexes). Mimic substances are such substances which simulate the prosthetic group and for example catalyze oxidation reactions.

In addition NaOCl can be added to the reaction mixture. This compound can form singlet oxygen in conjunction with hydrogen peroxide.

Finally it is possible to employ detergents. As those, non-ionic, anionic, cationic and amphoteric detergents are considered. The detergents can improve the penetration of enzymes and mediators in the fibre.

In addition it can be beneficial for the reaction to add polysaccharides and/or proteins. Here glucanes, mannanes,

dextranes, laevanes, pectines, alginates or plant gum and/or polysaccharides derived by fungi or those produced by mixed culture with yeast have to be mentioned for polysaccharides in particular and gelantine and albumin have to be mentioned for proteins in particular. Those substances serve mainly as protecting colloids to enzymes.

Additional proteins which can be added are proteases such as pepsin, bromeline, papaine and so on. These have the purpose among others to achieve a better access to lignin by reduction of extensine C, a protein rich in hydroxyproline, which exists in wood.

As additional protecting colloids amino acids, simple sugars, oligomere sugars, PEG types of different molecular weight, polyethylene oxide, polyethylene imine and polydimethylsiloxane are considered.

The process in accordance with this invention cannot only be used for delignification (bleaching) of sulfate, sulfite, organosol, or other cellulose and for wood pulp, but either for the manufacture of cellulose or wood pulp (refiner pulp/groundwood) in general from wood and annual plants for example. For that purpose it should be ensured that the defibration is been done by common cooking processes and/or mechanical processes or under pressure (that means a very gentle treatment up to kappa numbers in the range of >50 kappa or >10 % containment of lignin).

For bleaching of cellulose and for the pulpation of cellulose, also, the treatment can be repeated several times, either after the washing and extraction of the treated material by NaOH or without those in between steps. This leads to considerably further reduced kappa numbers and to a significant enhancement in whiteness. In addition before the enzyme/mediator-treatment a O2-stage can be used or as already mentioned a acidic washing or Q-stage (chelating stage) can be carried out.

In the following the invention is clarified by examples:

Example 1: Enzymatic bleaching by N-hydroxyphthalimid and softwood sulfate cellulose

5 g atro cellulose (softwood removed of lignin by O_2), pulp density 30% (about 17 g damp) are added to following solutions:
A) 20 ml tap-water are mixed with 30 mg N-hydroxyphthalimide (HPI) while stirring, pH-value has to be adjusted by 0.5 mol/l H_2SO_4-solution so that a pH 4.5 results after addition of enzyme.
B) 5 ml tap-water are mixed with such amount of laccase of *trametes versicolor* that an activity of 35 U (1 U = conversion of 1 µmol ABTS/min/ml enzyme) per g cellulose results.
Solutions A and B are mixed and filled up to 33 ml.
After addition of cellulose it is mixed by a dough kneading machine for 2 min.
After that the pulp is put into a pre-heated to 45 °C reaction bomb and is incubated under a O_2-pressure of 1 - 10 bar for 1 - 4 hours.
After that the pulp is washed over a nylon filter (30 µm) and extracted for 1 hour at 60 °C, 2% pulp density and 8% NaOH per g cellulose (atro).
After washing the pulp once again the kappa number is identified. The results are listed in table 1.

Example 2: Enzymatic bleaching by N-hydroxyphthalimid and hardwood sulfate cellulose

5 g atro cellulose (hardwood), pulp density 30% (about 17 g damp) are added to following solutions:
A) 20 ml tap-water are mixed with 30 mg N-hydroxyphthalimide (HPI) under while stirring. The pH-value has to be adjusted by 0.5 mol/l H_2SO_4-solution so that a pH 4.5 results after addition of enzyme.

B) 5 ml tap-water are mixed with such amount of laccase of *trametes versicolor* that an activity of 35 U (1 U = conversion of 1 µmol ABTS/min/ml enzyme) per g cellulose results.
Solutions A and B are mixed and filled up to 33 ml.
After addition of cellulose it is mixed by a dough kneading machine for 2 min.
After that the pulp is put into a pre-heated to 45 °C reaction bomb and is incubated under a O_2-pressure of 1 - 10 bar for 1 - 4 hours.
After that the pulp is washed over a nylon filter (30 µm) and extracted for 1 hour at 60 °C, 2% pulp density and 8% NaOH per g cellulose (atro).
After washing the pulp once again the kappa number is identified. The results are adduced listed in table 1.

Example 3: Enzymatic bleaching by N-hydroxymaleimid and softwood sulfate cellulose

5 g atro cellulose (hardwood removed of lignin by O_2), pulp density 30% (about 17 g damp) are added to following solutions:
A) 20 ml tap-water are mixed with 21 mg N-hydroxymaleimide (HPI) under while stirring, pH-value has to be adjusted by 0.5 mol/l H_2SO_4-solution so that a pH 4.5 results after addition of enzyme.
B) 5 ml tap-water are mixed with such amount of laccase of *trametes versicolor* that an activity of 35 U (1 U = conversion of 1 µmol ABTS/min/ml enzyme) per g cellulose results.
Solutions A and B are mixed and filled up to 33 ml.
After addition of cellulose it is mixed by a dough kneading machine for 2 min.
After that the pulp is put into a pre-heated to 45 °C reaction bomb and is incubated under a O_2-pressure of 1 - 10 bar for 1 - 4 hours.

After that the pulp is washed over a nylon filter (30 µm) and extracted for 1 hour at 60 °C, 2% pulp density and 8% NaOH per g cellulose (atro).
After washing the pulp once again the kappa number is identified. The result is adduced listed in table 1.

Table 1

results example 1 to 3

system	kappa before extraction	kappa after extraction	reduction of lignin [%]
zerovalues softwood O_2	9.6	9.3	3.1
HPI + softwood	7.8	6.7	30.2
zerovalues hardwood	12.7	11.5	9.5
HPI + hardwood	10.8	9.4	26
zerovalues softwood O_2	9.6	9.3	3.1
N-hydroxy-maleimide + softwood	9.1	8.1	15.6

Results refer to an incubation-span of 4 hours.

Example 4: Hydrolysis of N-hydroxyphthalimide in water (self-reducting of the mediator)

In one set-up 30 mg N-hydroxyphthalimide (HPI) are dissolved in 50 ml tap-water and adjusted to pH 4.5 by 0.5 mol/l H_2SO_4-solution. This solution is stirred at a temperature of 45 °C for 5 hours.
After the stated incubation-span the used HPI is 30% chemically changed to phthalic acid and hydroxylamine. Pthalic acid and hydroxylamine are produced in same molar proportion.

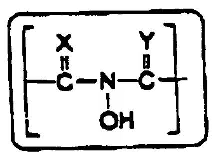
Claims

1. A multicomponent system for changing, reducing or bleaching of lignin, lignin-containing materials or similar substances containing

a. (if applicable) at least one oxidizing catalyst and

b. at least one suitable oxidizing agent and

c. at least one mediator characterized in so that the mediator is chosen from the group of cyclic N-hydroxy compounds with at least one (if applicable) substituted five- or sevenmembered ring containing the structure called formula A



formula A

as well as their salts, ethers or esters, where

X and Y are the same or different and represent O, S or NR^1, where

R^1 represents hydrogen-, hydroxy-, formyl-, carbamoyl-, sulfo-residue, ester or salt of the sulfo residue, sulfamoyl-, nitro-, amino-, phenyl-, aryl-C_1-C_5-alkyl-, C_1-C_{12}-alkyl-, C_1-C_5-alkoxy-, C_1-C_{10}-carbonyl-, carbonyl-C_1-C_6-alkyl, phospho-, phosphono-, phosphonooxy-residue, ester or salt of the phosphonooxy ester,

where carbamoyl-, sulfamoyl-, amino- and phenyl-residues can be unsubstituted or once or multiple substituted with a residue R^2 and the aryl-C_1-C_5-alkyl-, C_1-C_{12}-alkyl-, C_1-C_5-alkoxy-, C_1-C_{10}-

carbonyl-, carbonyl-C_1-C_6-alkyl-residues can be saturated or unsaturated, branched or unbranched and can be substituted once or multiple by a residue R^2 where

R^2 is the same or different and represents hydroxy-, formyl-, caboxy-residue, ester or salt of the carboxy residue, carbamoyl-, sulfo-ester or salt of the sulfo residue, sulfamoyl-, nitro-, amino-, phenyl-, C_1-C_5-alkyl-, C_1-C_5-alkoxy-residue.

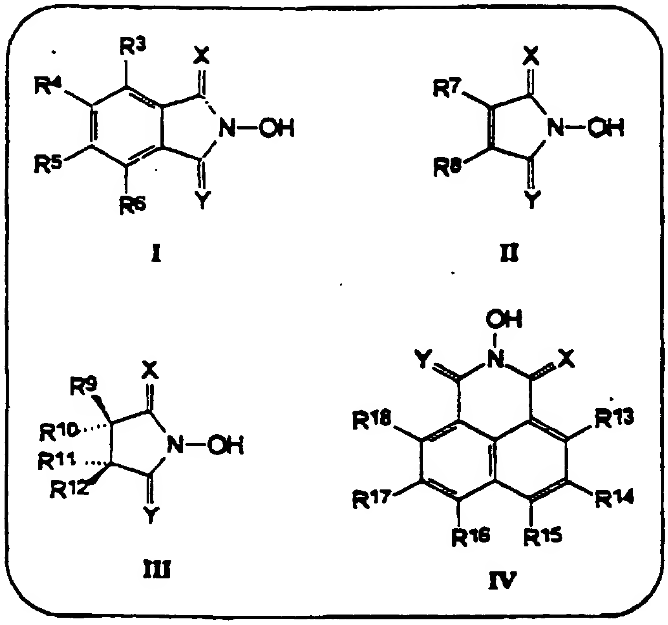
2. A multicomponent system in accordance with claim 1, characterized so that at least one oxidizing catalyst is used.

3. A multicomponent system in accordance with claim 1 or 2, characterized so that enzymes are used as oxidizing catalyst.

4. A multicomponent system in accordance with one of claim 1 to 3, characterized so that laccase is used as enzyme.

5. A multicomponent system in accordance with one of claim 1 to 4, characterized so that air, oxygen, ozone, H_2O_2, organic peroxides, peracids such as peracetic acid, performic acid, persulfuric acid, pernitric acid, metachlorineperoxibenzoic acid, perchloride acid, perborates, persulfates, peroxides or oxygen species and their radicals such as $OH^{\bullet}$, $OOH^{\bullet}$, singlet oxygen, superoxide $(O_2^{\bullet-})$, ozonide, dioxygenyl-cation (O_2^{+}), dioxiranes, dioxetanes or Fremy radicals can be used as oxidizing agent.

6. A multicomponent system in accordance with one of claim 1 to 5, characterized so that at least one compound of the general formula I, II, II or IV is used as mediator (component c),

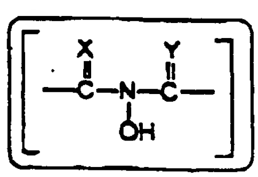


where X, Y, have the meanings previously mentioned and the residues R^3-R^{18} are the same or different and the halogen residue, carboxy residue, salt or ester of a carboxy residue or the meanings mentioned for R^1,

where R^9 and R^{10} respectively R^{11} and R^{12} can not represent hydroxy- or amino-residue at same time and

(if applicable) each two of the substitutes R^3-R^6, R^7-R^8, R^9-R^{12}, R^{13}-R^{18} can be connected to a ring -B-, where -B- has one of the following meanings:

$(-CH=CH)-_n$ with n = 1 to 3, -CH=CH-CH=N- or



formula A

and where (if applicable) the residues R^9-R^{12} can be connected by one or two bridging elements -Q-, where -Q- is the same or different and have one of the following meanings: -O-, -S, $-CH_2-$, $-CR^{19}=CR^{20}-$;

where R^{19} and R^{20} can be the same or different and have the same meaning as R^3.

7. A multicomponent system in accordance with one of claim 1 to 6, characterized so that at least one substance is used chosen from the group N-hydroxy-phthalimide, (if applicable) substituted N-hydroxy-phthalimide-derivatives, N-hydroxymaleimide, (if applicable) substituted N-hydroxymaleimide-derivatives, N-hydroxy-naphthal acid, imide-derivatives,N-hydroxylsuccinimide-derivatives are used as mediator.

8. A multicomponent system in accordance with one of claim 1 to 6, characterized so that N-hydroxypthalimide is used as mediator.

9. A Process for treatment of lignin, characterized so that the particularly chosen components a) to c) in accordance with claim 1 are mixed simultaneously or in arbitrary order with an aqueous suspension of lignin-containing materials.

10. A use of mediators in accordance with claim 1 for changing, reducing or bleaching of lignin, lignin-containing materials or similar substances.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 97/01546

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 D21C9/10 D21C5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
IPC 6 D21C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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X	DATABASE WPI Section Ch, Week 9616 Derwent Publications Ltd., London, GB; Class A41, AN 96-155414 XP002036111 & JP 08 038 909 A (DAICEL CHEM IND LTD) , 13 February 1996 see abstract ---	1,2,5-8
A	WO 94 29510 A (CALL HANS PETER) 22 December 1994 cited in the application see page 11; claims ---	1-5,8,9
A	US 5 478 356 A (KAARET THOMAS W) 26 December 1995 see the whole document ---	1-3,5-10

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
25 July 1997	11.08.97

Name and mailing address of the ISA	Authorized officer
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Inter[nation]al Application No
PCT/EP 97/01546

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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